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SOME OBSERVATIONS ON THE ANATOMY OF *ARTEMISIA AFRA* JACQ.

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Abstract

The anatomy of *Artemisia afra* was investigated at the light microscope level, using fresh, paraffin and Spurr's-embedded stem, petiole, rachis, petiolule and leaflet material. The young stem consists of a uniseriate epidermis, a cortex of collenchyma and parenchyma, a poorly defined multilayered starch sheath and a ring of collateral vascular bundles surrounding an entirely parenchymatous pith. The primary phloem contains thick-walled sieve tubes. Oil ducts are spatially associated with the vascular bundles and starch sheath.

Artemisia afra has pinnatisect leaves. The main vein is surrounded by a poorly defined multilayered parenchymatous sheath. As in the stem, the phloem of the main vein contains thick-walled sieve tubes. A pair of adaxially situated oil ducts occur within the bundle sheath of the main vein. The anatomy of the rachis is similar to that of the petiole. The midrib of the petiolule contains a single adaxial oil duct, which is located within the bundle sheath. Similarily, the main vein of the leaflet is associated with a single adaxial oil duct. No oil ducts are associated with the second and third order veins of mature leaflets. The minor veins of the leaflets contain phloem transfer cells.

Uittreksel

WAARNEMINGS OOR DIE ANATOMIE VAN *ARTEMISIA AFRA* JACQ.

Die anatomie van *Artemisia afra* is ondersoek op die ligmikroskoop vlak. Dwarsdeursneë van die stingel, petiool, ragis, petioluul en pinna in was en plastiek ingebed sowel as vars materiaal is gebruik. Radiale lengtesneë is ook van die stingel gemaak. Die jong stingel word begrens deur 'n epidermis waaronder 'n buitenste kortekslaag van kollenchiem en 'n binneste kortekslaag van parenchiem voorkom. Die kollaterale vaatbondels word na buite begrens deur 'n swak gedefinieerde meerlagige setmeelskede. Die murg is geheel-en-al parenchimaties. Die floeëm van hierdie spesie bevat dikwandige sifvate. Oliekanale met omringende sekreetselle is met die vaatbondels en setmeelskede geassosieer.

Artemisia afra het gepinnatisekteerde blare. Die hoofaar van die goed ontwikkelde petiool word deur 'n swak ontwikkelde meerlagige parenchimatiese bondelskede omgewe. Soos in die stingel bevat die floëem van die hoofaar ook dikwandige sifvate. Twee adaksiale oliekanale is geassosieer met die bondelskede van die hoofaar voor. Die anatomie van die ragis stem ooreen met dié van die petiool. Die hoofaar van die petioluul bevat 'n enkele adaksiaal geleë oliekanaal, ingesluit deur die bondelskede. Netso bevat die hoofaar van die pinna ook 'n enkele adaksiaal geleë oliekanaal binne in die bondelskede. Geen oliekanale kom voor of is geassosieer met die tweede en derde orde are van volwasse pinnas nie. Floeëm oordragselle is waargeneem in die kleiner aartjies van die pinnas.

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INTRODUCTION

Artemisia afra Jacq., (Tribe : Anthemideae) commonly known as wormwood or wilde-als, is a member of the Compositae. *Artemisia* ranges from Ethiopia, through Eastern Tropical Africa, down into Southern Africa. *Artemisia* is widely distributed in the eastern part of South Africa, extending as far south as Stellenbosch (Hilliard, 1977).

Artemisia species have long been recognised as sources of essential oils (Brouk, 1975; Guenther, 1952; Sievers, 1947; Schery, 1972). *Artemisia afra* is one of the plants currently being studied as a source of essential oils in the Faculty of Agriculture at Fort Hare University.

The primary aim of this study was to examine the anatomy of the aerial parts of *Artemisia afra*, with a view to locating the oil ducts and their associated secretory cells. During this study, material containing only primary vascular tissues was used in determining the location of the oil ducts.

MATERIAL AND METHODS

All plant material used in this study was harvested in the field and immediately transferred to our laboratories for further processing. Suitable young and mature stem segments, petiole, rachis, petiolule and entire leaflets were fixed in either FAA (Sass, 1958) or in 4% glutaraldehyde in 0,05M phosphate buffer, pH 7,0. Material fixed in FAA was dehydrated in a TBA series, and embedded in paraplast wax and sectioned at 10 μm on a Leitz rotary microtome. Glutaraldehyde-fixed material was dehydrated in an alcohol series and embedded in Spurr's resin. Serial sections were cut at 0,5 to 2,0 μm, with glass knives on an LKB Ultrome II. Wax embedded serial sections were stained in safranin fast green-gentian violet (Johansen, 1940) and mounted under coverslips in DPX synthetic mounting medium. Plastic sections were viewed either unstained, or stained in 0,05% toluidine blue 0, pH 7,0 for 5 min on a hotplate (O'Brien, Feder and McCully, 1964) or in 0,05% fluorecin-K in distilled water, for 20–30 min. All plastic sections were mounted in Entellan rapid mounting medium (Merk).

For localisation of oil ducts and confirmation of the presence of oils within the ducts, fresh material was sectioned on a Leitz freezing microtome at 15–20 μm and stained in Sudan IV (0,5% w/v in 95% ethyl alcohol) according to the procedure outlined in Johansen (1940). Selected sections were photographed with a Zeiss photomicroscope III, using brightfield, phase-contrast, and transmitted fluorescence optics.

RESULTS AND DISCUSSION

1. *Stem*

The ribbed stem of *Artemisia afra* is bounded by a single-layered epidermis. The outer cortex consists of lamellar collenchyma (Fig. 1) which is especially well developed beneath the ribs. The inner cortex consists of mixed parenchyma-chlorenchyma tissue and a poorly defined starch sheath, 1–2 cells in width (SS, Figs 1–2). The primary vascular bundles are collateral and are closely spaced. In Figures 1–2, the primary xylem and primary phloem are separated by a fascicular cambium, which apparently has not yet produced any secondary vascular tissues.

The phloem consists of sieve tubes and associated parenchymatous cells, including companion cells. Metcalfe and Chalk (1950) have reported that the phloem of Compositae sometimes contain lignified elements. During the present investigation, thick-walled sieve tubes were observed in stem and leaf material. These thick-walled sieve tubes are very conspicuous in transections stained with fluorecin-K, and viewed with fluorescence optics (Fig. 1). Judged by the relative fluorescence intensities, the sieve tubes are not lignified, but have thick cellulosic cell walls (compare rather weak fluorescence of walls of lignified xylem elements with strong fluorescence of walls of collenchyma and sieve tubes in Fig. 1). Further, when stained in toluidine blue O, the walls of the sieve elements typically were blue to purple in colour and not green as would be the case if they were lignified.

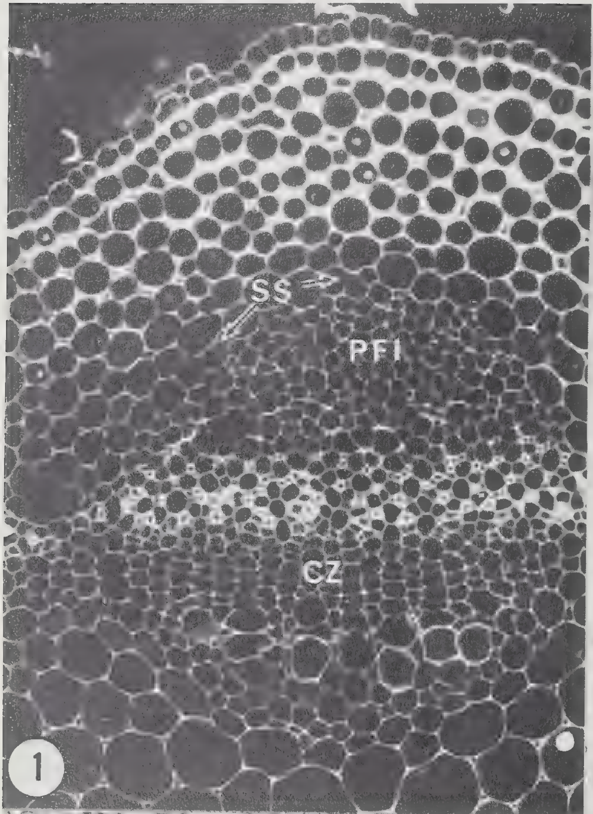
As mentioned, the vascular bundles illustrated in Figures 1–2 apparently are composed entirely of primary tissues. No secondary vascular tissues are evident. In such material the interfascicular regions are parenchymatous, with the exception of isolated patches of phloem elements, including thick-walled sieve tubes (PE, Fig. 2). Figure 2 shows an oil duct surrounded by four secretory cells (open circles) just beneath the starch sheath. Note that the walls of the secretory cells are more darkly stained than those of the surrounding parenchymatous cells. The oil duct is occluded with a densely stained substance. When viewed in tangential section (Fig. 3) the relationship of the relatively narrow oil duct to its surrounding secretory cells is clear. Three amorphous deposits are visible in this duct (unlabelled arrows). Similar sections stained in Sudan IV indicated that these deposits could be some form of oil.

Oil ducts and their associated secretory structures may arise within the plant either by lysigeny or schizogeny, or by a combination of both processes (Esau, 1965). Examination of transverse, tangential and radial longitudinal sections revealed that the ducts in the *Artemisia* stem are schizogenous in origin, that is, that they arise by schizogenous separation of the inner walls of the secretory cells.

2. *Petiole, Rachis, Petiolule and Leaflet*

.1 Leaf Architecture

In order to gain a more thorough understanding of the architecture of the compound leaf, whole leaves were cleared (Foster, 1952) and examined under the microscope. Venation of the individual leaflets is simple and, according to the classification system of Hickey (1973), brochidodromous. Each leaflet contains a single primary vein, from which a number of secondaries emerge.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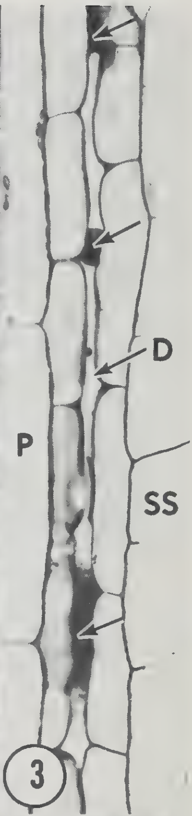
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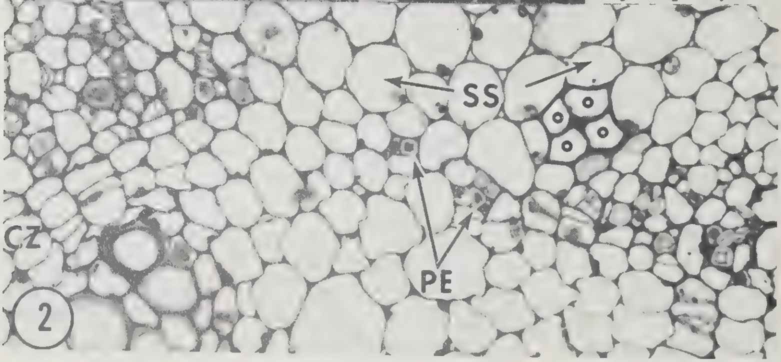
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The third order veins are weakly developed and few in number. Anatomical investigation indicated that the second and third order veins constitute the minor veins in leaflets of the *Artemisia* leaf. Areolation shape was found to be variable, but mostly quadrangular.

.2 Anatomy

.2.1 Petiole

As viewed in transection, the petiole consists of a single large crescentric collateral vascular bundle surrounded by parenchymatous ground tissue. The vascular bundle is delimited by a poorly defined parenchymatous sheath. As in the stem, the phloem contains thick-walled sieve tubes. Each midrib vascular bundle examined was associated with two adaxially situated oil ducts which were spatially associated with the bundle sheath (Fig. 4). As with the stem bundles, the midrib vascular bundle is surrounded by a multilayered sheath (Fig. 4).

.2.2 Rachis

The anatomy of the rachis, as one might expect, was similar to that of the petiole (Fig. 4). Figure 6 shows one of two oil ducts situated beneath the bundle sheath of the main vein of the rachis. Fibres occur above and below this vascular bundle. Figure 5 shows part of a lateral vein of a rachis-petiole region. This vein has a single oil duct located above the protoxylem but beneath the bundle sheath. This vein constitutes the main vascular supply of the leaflet. As in the rachis (Fig. 6), fibres occur above and below the vascular bundles.

FIG. 1.

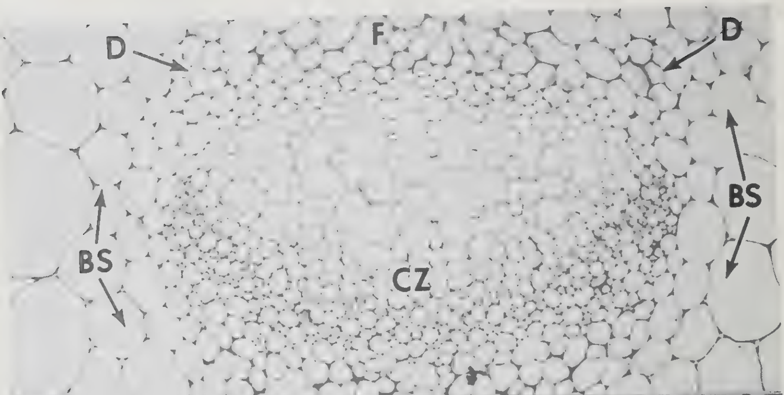
Fluorescence photomicrograph of a transection of part of a young stem. Note relatively weak fluorescence of walls of parenchymatous cells, including those of the cambial zone (CZ) and immature primary phloem fibres (PFI). Starch sheath (SS) delimits vascular from ground tissue. x 300.

FIG. 2.

Transection of a young stem, showing part of two adjacent primary vascular bundles. Some metaxylem elements are mature. Thick-walled cells immediately above the cambial zone (CZ) are sieve tubes. A poorly defined starch sheath (SS), delimits vascular from ground tissue. An oil duct, surrounded by four secretory cells (open circles) is associated with the bundle on the right. Phloem elements (PE) between bundles have thick walls, as do the metaphloem elements within the bundles. x 720.

FIG. 3.

Tangential section, showing part of an oil duct (D) similar to that shown in transection in Figure 2. The oil duct contains darkly stained material, presumably oil. The secretory cells are bordered by parenchymatous cells (P) to the left, and starch-sheath cells (SS) to the right. x 720.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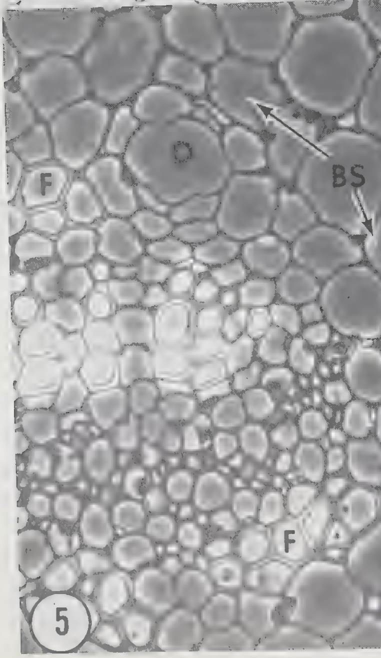
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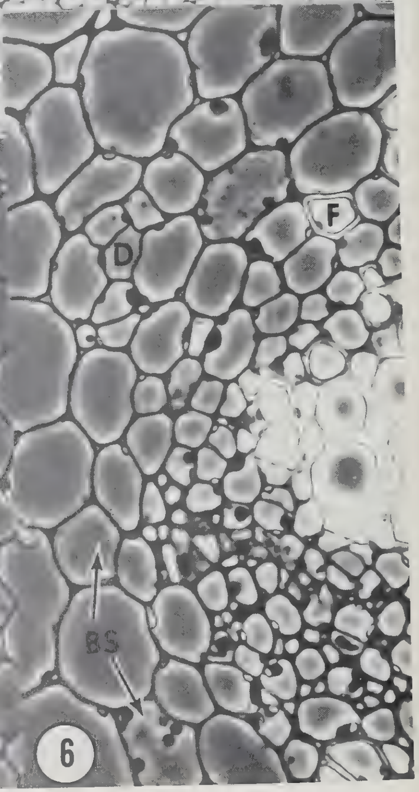
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.2.3 Petiolule and Leaflet

The petiolule of each leaflet of *Artemisia* is winged and contains a large, acentrically situated main vein, together with associated minor veins. Figures 7–8 are transections of the same main vein at two levels of the leaflet. Of interest is the fact that the bundle sheath contains fairly conspicuous chloroplasts. Figure 7 shows the arrangement of the vascular tissues and the single adaxial oil duct near the base of the leaflet; Figure 8 near the tip of the leaflet. Oil ducts were not found in veins smaller than the one shown in Figure 8.

During the investigation, it became apparent that the remaining two orders of veins (i.e. second and third orders) contained parenchymatous cells, with conspicuously thickened cells walls. These parenchymatous cells were associated with the sieve tubes. Figure 9 shows a third order vein with two sieve tubes and two tracheary elements. At this magnification, the wall thickenings of neighbouring parenchymatous cells are discernible. Moreover, fluorescence microscopy revealed that these cells are transfer cells. Figure 10 shows two sieve elements surrounded by 5 transfer cells with prominent wall ingrowths. Note that the wall ingrowths are more conspicuous on the cell walls adjacent to the sieve tubes than elsewhere.

Transfer cells are not uncommon in the Compositae. Gunning, Pate and Green (1970) list 13 genera in which transfer cells have been positively identified. The transfer cells of minor veins have been implicated in the retrieval and transfer of solutes to the sieve tubes, from both the transpiration stream and the pathways of photosynthates (Pate and Gunning, 1972). Gunning and Pate (1974) and Gunning, Pate and Briarty (1968) have suggested that the initiation of wall ingrowths in minor veins coincides with, or slightly precedes commencement of export of photosynthates from the leaf. Further development of wall ingrowths parallels build-up in export activity.

FIG. 4.

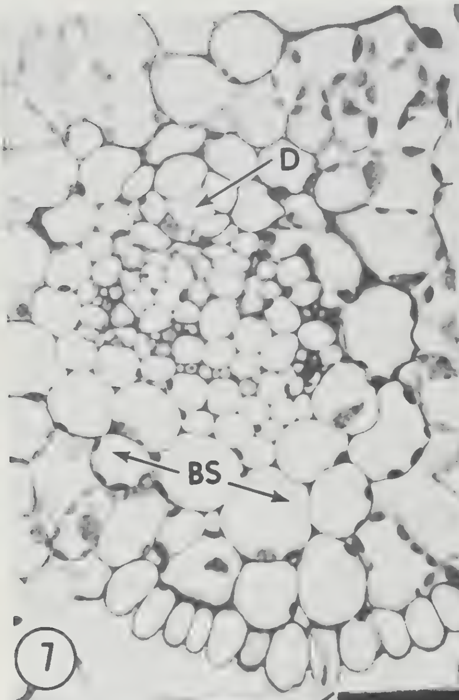
Transverse section of petiole, showing crescentric collateral vascular bundle. Bundle sheath (BS) is poorly differentiated and lacks chloroplasts. The xylem and phloem apparently are entirely primary, although separated by a cambial zone (CZ). Two adaxially located oil ducts (D) can be seen inside the bundle sheath. The vascular bundle is capped by fibres (F) above and below. x 290.

FIG. 5.

Phase contrast photomicrograph of a lateral vein in the rachis—petiolule region. This vascular bundle contains a single adaxial oil duct (D), surrounded by several secretory cells. Fibres (F) occur above and below the vascular bundle. The vascular bundle, including the oil duct and its associated secretory cells, is ensheathed by a parenchymatous bundle sheath (BS). x 720.

FIG. 6.

Phase contrast photomicrograph showing part of the main vein of the rachis. One of two adaxial oil ducts (D) is visible. As in Figure 5, the vascular tissue is bordered by fibres (F) and surrounded by an achlorophyllous parenchymatous bundle sheath (BS). x 720.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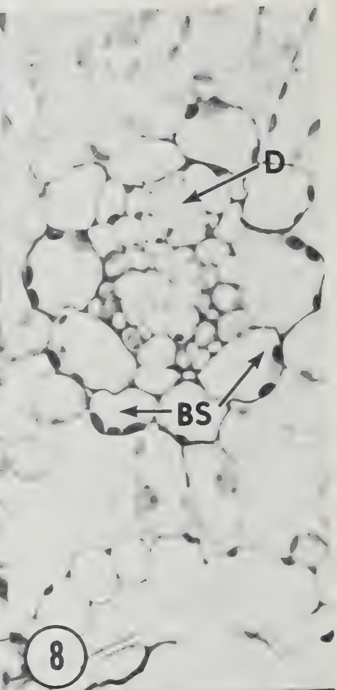
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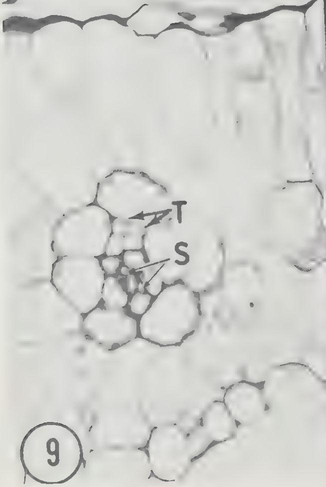
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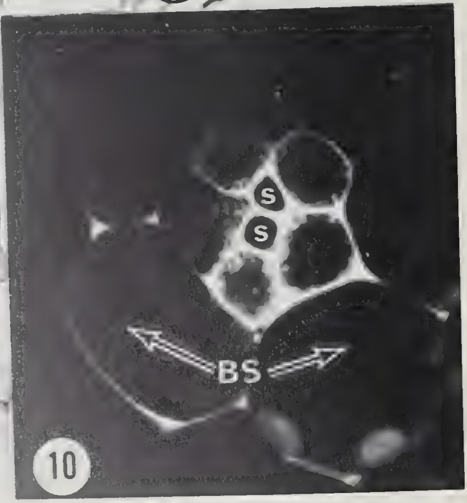
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CONCLUSION

During the investigation oil ducts were localized in young stem and leaf material. These oil ducts were associated with the primary vascular bundles in the stem and with primary, or first order veins of the petiole, petiolule and leaflets, but not with the minor veins. It is possible that these oil ducts terminate with the primary vein system in the leaflets of *Artemisia*.

The phloem of this species requires further mention, due to the presence of thick-walled, apparently non-lignified sieve elements in stem and leaf material and the association of minor vein sieve elements with transfer cells. The nature of the sieve element cell wall and the structural relationship of sieve elements to transfer cells in minor veins are currently being investigated.

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FIG. 7.

Transection of main vein near base of leaflet. An oil duct is located immediately above the protoxylem. Bundle sheath (BS) contains chloroplasts. x 640.

FIG. 8.

Transection of main vein near tip of leaflet. As in Figure 7, the oil duct is located adaxial to the protoxylem. Bundle sheath (BS) contains chloroplasts. x 640.

FIG. 9.

Transection of minor vein of leaflet, with two sieve tubes (S) and two tracheary elements (T). Bundle sheath cells have chloroplasts. Thickenings of the phloem parenchyma cell walls are discernible at this magnification. x 640.

FIG. 10.

Fluorescence photomicrograph of minor vein of leaflet, showing two elements (S) surrounded by transfer cells with prominent wall ingrowths are most strongly developed adjacent to the sieve elements. Tracheary elements are not discernible in this micrograph, due to weak fluorescence of their walls. x 900.

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